

# Invasion of the noxious weed *Nicotiana glauca* R. Graham after an episodic flooding event in the arid zone of Australia

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## 1. Introduction

Natural disturbances such as flooding and wind facilitate further extent of already established exotic species into new areas (Cellot et al., 1998; Williams and West, 2000; Varnjic et al., 2000). Virtue et al. (2001) suggested exotic species can invade into a new territory by several ways: incidental introduction by human influenced means, deliberate introduction, accidental movement, wind, water, and wild terrestrial vertebrates. Sometimes, native plant species rely on such events for their recruitment, growth and reproductive. In particular, in arid landscapes, high rainfall driven flooding events may occur only once in hundred years. These sporadic and unpredictable flooding events facilitate irruption of larger numbers of native annual and perennial species from the soil seed bank (Westbrooke et al., submitted). However, invasive taxa make use of such rare events to extend their territory. One such event occurred in Olary Creek in February 1997, after 216 mm of rainfall was received in 24 h in the Olary Ranges, South Australia. This high rainfall created a heavy flood and formed a terminal lake in Nagaela station, New South Wales. Although some studies have been carried out on high rainfall flooding events and their impacts on native species (Weston and Westbrooke, 1999; Florentine et al., 2003; Westbrooke et al., submitted), no work has been done on the impact of rare flooding event and exotic invasion of arid landscape in Australia.

*Nicotiana glauca*, a fast growing shrub or small tree, native to South America, belongs to the family Solanaceae (Mizrachi et al., 2000). It branches profusely, and can grow vigorously to 3 m, particularly after good rainfall events. Flowering commences approximately one year after germination and a fully-grown plant can produce 10,000–1,000,000 seeds. The most effective mode of long-distance seed dispersal is through hydrochory. *N. glauca* is found in open and disturbed areas including wastelands, roadsides and creek lines (Boyland et al., 1985). Extensive stands may be found for some years on stream floodplains and drainage channels after summer floods (Cunningham et al., 1981). Studies have demonstrated that *N. glauca* is highly toxic to humans (Mizrachi et al., 2000) and animals (Panter et al., 2000). The species is usually avoided, as it is unpalatable; however during drought, when food resources are scarce, livestock may consume the plant and die (Cunningham et al., 1981). The plant contains the alkaloid anabasine, which is considerably more toxic than nicotine (Sims et al., 1999). Panter et al. (2000) found that *N. glauca* causes defects in foetal goats and sheep if the mother does not die from consumption of the plant.

Although a number of studies have been carried out on the toxicity of this plant in America and Germany, little research has been conducted in Australia. Extensive

growth of *N. glauca* was observed around a lake, formed following a rare flood event in Olary Creek in 1997. The main objectives of this study were to investigate: (i) the distribution of *N. glauca* in relation to the 1997 flood event; (ii) the variation in *N. glauca* soil-stored seed densities between soil in control and exclosure plots and (iii) the allelopathic potential of *N. glauca* litter on germination of *Lactuca sativa* seeds.

## 2. Materials and methods

### 2.1. Study area

The study was conducted on Nagaela Station, around a lake formed in 1997 following a flood of Olary Creek. The ephemeral Olary Creek arises near Olary in the north east of South Australia. The creek flows south and east through South Australia to Oakvale Station. There it splits into two channels, one of which flows due east into New South Wales to fill Woolcunda Lake and White Lake, and the other south-east through Loch Lilly Station. The Creek flowed for the first time for many years in February 1989 and again, more extensively, in February 1997 following high rains in the catchment (Weston and Westbrooke, 1999). Following this event the southern arm flowed on into Nagaela Station where it formed a new lake of approximately 40 ha within mallee shrubland (Fig. 1). The lake gradually dried over a two-year period. Weston and Westbrooke (1999) indicate that the creek flowed only four times between 1921 and 1997. The flood in 1997 was the most extensive on record and followed a path to fill a lake not flooded since settlement. Preliminary studies at the site have helped to explain previously observed examples of perennial vegetation regeneration difficult to attribute to on-site rainfall events. The creek provides a model for many other such ephemeral creek systems across arid Australia.

### 2.2. Chronological events

Rare flooding event occurred in September 1997. Twenty months after flooding in November 1999 plots were established in the flooded and unflooded (control) area as the lake dried. One of the plots in the pair was left unfenced while the other was fenced to prevent grazing by vertebrate animals. Detailed vegetation data were collected in October 2000 (3 years and 10 months after the flooding event started) and October 2002 (5 years and 10 months after flooding event started).

### 2.3. Distribution of *N. glauca* in relation to 1997 flooding event

To assess the effects of the 1997 flood on native flora, six  $25 \times 25 \text{ m}^2$  exclusion plots were established on flooded (4) and non-flooded control (2) areas. Adjacent to each plot (fenced to exclude rabbits, goats and kangaroos), a control plot (located in close proximity to treatments plots, in the same ecological vegetation class and land systems) was also established by marking four corners with wooden stakes. The fence

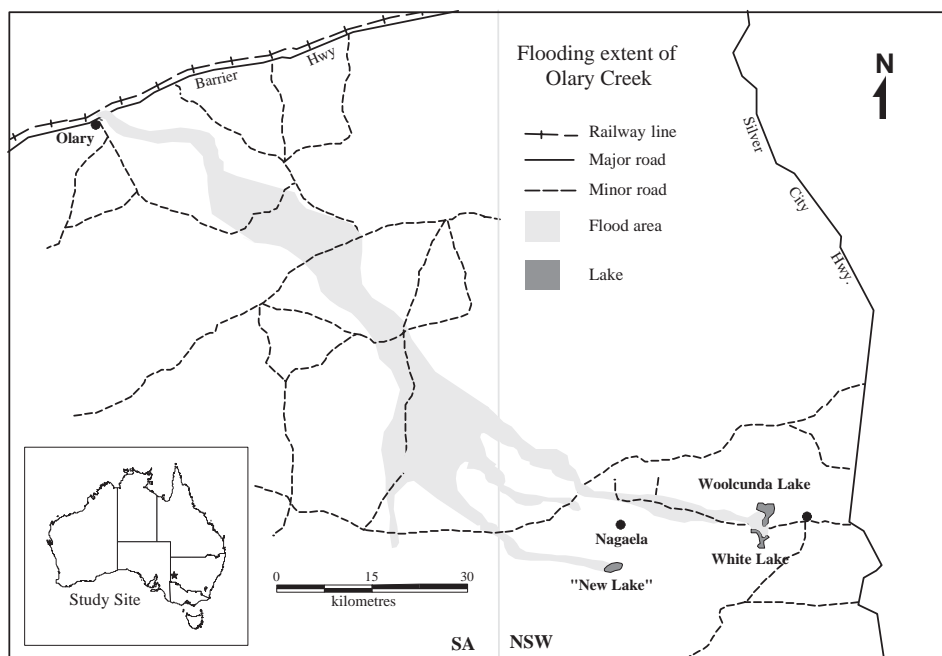


Fig. 1. Location of study site and extent of the 1997 Olary Creek flooding.

was 1.7 m tall, with a 150 mm mesh size and rabbit proof mesh to 80 mm. The data presented here focuses on the response of *N. glauca* to the flood event. Plots were assessed in September 1998, October 2000 and October 2002. Each  $25 \times 25 \text{ m}^2$  plot was divided into 25,  $5 \times 5 \text{ m}^2$  sub plots. Within each sub plot, all *N. glauca* species were located, their heights, diameter (20 cm from the soil surface), and whether the plants were dead or had resprouted was recorded.

#### 2.4. Density distribution from the lake boundary

Cunningham et al. (1981) reported that *N. glauca* grows on floodplains and drainage channels and is less prevalent away from creek lines. To examine the pattern of *N. glauca* distribution around the “new lake”, belt transects ( $294 \times 3$  and  $228 \times 3 \text{ m}^2$ ) were established running north and south from the lake centre. These belt transects were divided into three zones: long-term flood zone (approximately 1–1.5 m in depth flood water stayed approximately 20 months), short-term flood zone (approximately 30–50 cm depth floodwater stayed less than a year) and non-flood zone (just after the short term flood zone but far away from the control plots), based on the flooding mark. Each transect was divided further in to  $3 \times 3 \text{ m}^2$  sub-plots. Within each sub-plot the height (m) and diameter (cm) at 20 cm for all *N. glauca* were recorded. *N. glauca* used the 1997 rare flooding event to establish and

survive, but during subsequent years of new average rainfall, *N. glauca* shed leaves and shoot tips dried out. However, during 2002 measurements, significant number of *N. glauca* plants started to resprout. Resprouting from the branch or base (close to root collar) or from both branches and base were recorded.

## 2.5. Dendrochronology

To link the 1997-flooding event with *N. glauca* recruitment five single stem trees of largest stem diameter were marked. These trees were cut down and sections cut approximately 1 m from the base. Sections were mounted and fine-sanded. Growth rings were counted on each sample under an Olympus<sup>TM</sup> (Model SZ-PT Sz40) compound microscope following the technique used by Hughes (1992). The dendrochronology study described incorporates two assumptions. Firstly, *N. glauca* produce one growth ring/year, secondly, sufficient samples are taken to cover local variation. Westbrooke (1999) confirmed annual growth ring in *Myoporum platycarpum* ssp. *platycarpum* growing in the same area.

## 2.6. Soil-stored seed bank

Soil samples were collected in October 2002. Twenty samples were collected from randomly selected points from each control and exclosure plot. Random 10 × 10 cm<sup>2</sup> quadrats were selected and soil was removed with a small trowel to a depth of 15 cm. Samples were placed in separate labelled bags, transported to Ballarat and stored in a glasshouse (20–25 °C) for two days.

Individual samples were thoroughly mixed within the bag to ensure a uniform distribution between seeds and soil then spread evenly in 8.5 × 14.0 × 5.5 cm punnets lined with paper towel. Punnets were individually labelled with the sample number and placed in large trays (28.0 × 44.0 × 5.5 cm), which were placed on benches in a glasshouse. On each bench, a further tray containing sterilized soil placed randomly between soil seed sample trays was used as a control. All trays were watered twice a day. The germinable seed bank was considered as the number of seeds that germinated under favourable water and temperature conditions within 90 days (Bertiller, 1996). This measure provides an estimate of the immediately germinable seed bank. The number of germinated *N. glauca* seedlings was recorded weekly.

## 2.7. Allelopathic effects

An aqueous leachate of freshly collected *N. glauca* leaves, dry leaves, fresh bark and twigs (> 1 cm) was prepared by soaking 30 g of material in 240 mL of deionized water (1:8 by weight) in 500 mL flasks for 24 h at room temperature. The leachate was filtered into a labelled flask, using Whatman<sup>®</sup> filter paper (125 mm, No: 3) and stored at 5 °C until use. Following filtering, four concentrations were prepared: Treatment 1 (control) = 100% deionized water; Treatment 2 = 100% leachate, Treatment 3 = 75% leachate + 25% deionized water, Treatment 4 = 50%

leachate + 50% deionized water and Treatment 5 = 25% leachate + 75% deionized water.

Seeds of *L. sativa* L. cv. Great Lakes were selected to test the effects of leachate on germination, because of their uniform germination behaviour (Dietz and Winterhalter, 1996) and sensitivity to germination inhibitors (Evenari and Newman, 1952). Four replicates, each of 50 seeds, were placed in labelled Petri dishes lined with two No. 3 Whatman<sup>®</sup> filter papers. The filter paper was moistened with 5 mL of aqueous leachates of the respective solutions, while the control received the same amount of deionized water. Petri dishes were placed in a growth cabinet at 21 °C and kept moist until the experiment was terminated at 30 days.

## 2.8. Data analysis

Data were analysed using the Super ANOVA software program (Abacus Concepts, Berkeley, California) for obtaining two-way ANOVA and all pairwise multiple comparison procedures. Residual plots of each ANOVA were obtained to examine homogeneity of the variance. Data were then transformed log (stem diameter and height) or arcsign (*L. sativa* seed germination data) and reanalysed. Means were compared using Tukey's HSD test (Day and Quinn, 1989).

## 3. Results

### 3.1. *N. glauca* seedling emergence in relation to flooding event

*N. glauca* density varied between flooded fenced and unfenced plots and also varied between assessments. In September 1998, no *N. glauca* was found across flooded and control plots. In October 2000, densities of 24 and 12 *N. glauca* seedlings ha<sup>-1</sup> were recorded from flooded unfenced and fenced plots respectively. This number had significantly ( $p > 0.0001$ ) increased by October 2002, when 756 and 468 ha<sup>-1</sup> were recorded in the flooded unfenced and fenced plots respectively. *N. glauca* was not recorded from control fenced and unfenced plots (Fig. 2).

During 2002 measurement the flooded unfenced plots 80% of the plants were found resprouted, and of these, 90% and 10% of them were resprouted from the base and from branches respectively. Only 20% were found dead or not resprouted. Similarly in the flooded fenced plots 72% of plants resprouted, of these 87% and 13% resprouted from the base and branches respectively. Of 117 plants recorded 26% were either dead or not resprouted.

### 3.2. Dendrochronology

The dendrochronology study revealed a maximum of five growth rings. The dendrochronology study described incorporates two assumptions. Firstly, *N. glauca* produce one growth ring/ year, secondly, sufficient samples are taken to cover local

variation. Westbrooke (1999) confirmed annual growth ring in *Myoporum platycarpum* ssp. *platycarpum* growing in the same area.

### 3.3. Density distribution around the new lake

There was a clear pattern in *N. glauca* distribution. In the two transects a total of 366 trees were recorded. Larger numbers of trees ( $n = 283$ ) were recorded in the short-term flood zone followed by long-term flood zone ( $n = 53$ ) and non-flood zone ( $n = 8$ ) (Fig. 3A and B). A correlation analysis between diameter (cm) and height (m)

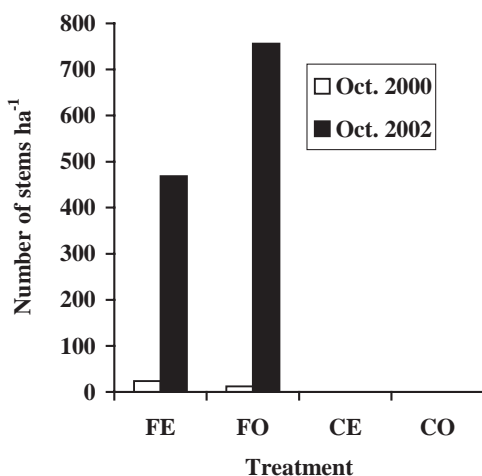


Fig. 2. *N. glauca* stem density in FE flood enclosure, FO flood open; CE control enclosure and CO control open.

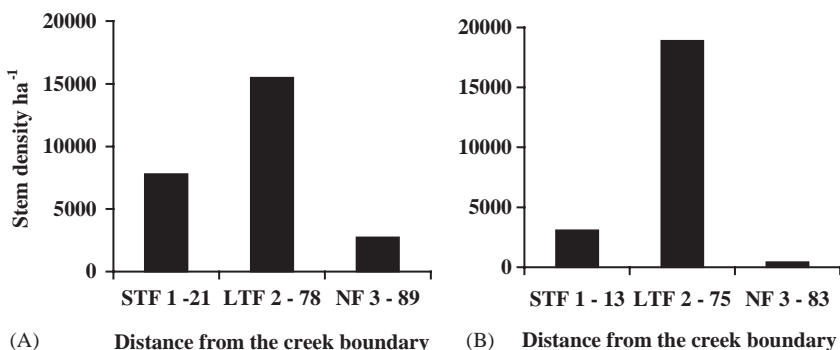


Fig. 3. *N. glauca* stem density distribution in relation to 1997 flood. Transect A, N S direction and B, S N direction. STF short term flood zone; LTF long term flood zone & NF no flood.

of *N. glauca* performed separately for both transects produced the following equations, for the two transects (Fig. 4A and B).

$$\text{Transect 1} \quad \text{Height} = 1.105 + 0.1142(R^2 = 0.85; p < 0.0001), \quad (1)$$

$$\text{Transect 2} \quad \text{Height} = 0.977 + 0.1794(R^2 = 0.73; p < 0.001). \quad (2)$$

A comparison of the  $R^2$  values for Eqs. (1) and (2) indicated no difference between transects.

Similarly correlation analysis between stem diameter (cm) and height (m) of *N. glauca* was performed on the data collected from the flooded open and enclosed plots (Fig. 5A and B). The following equations were produced.

$$\text{Open plots} \quad \text{Height} = 0.508 + 0.4368(R^2 = 0.29; p < 0.005), \quad (3)$$

$$\text{Enclosed plots} \quad \text{Height} = 0.757 + 0.0230(R^2 = 0.35; p < 0.003). \quad (4)$$

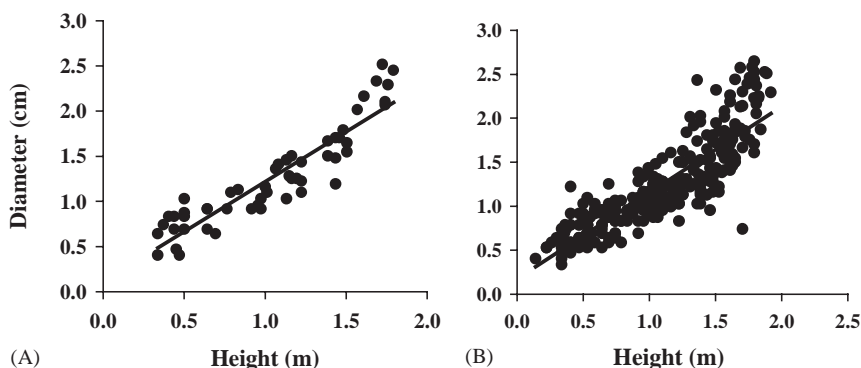


Fig. 4. The relationship between *N. glauca* height (m) and diameter (cm) measured in zone 1 (A) and 2 (B).

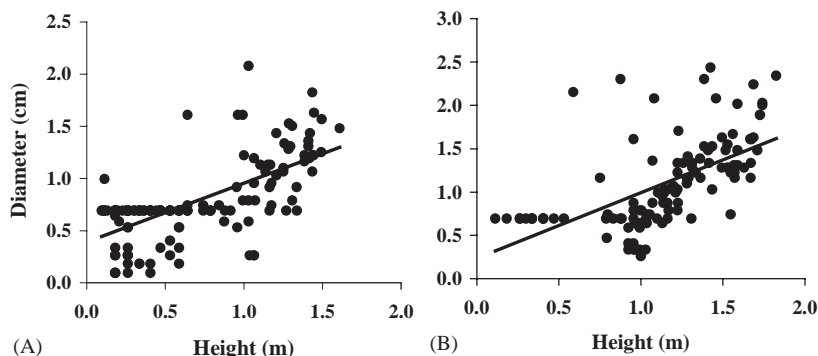


Fig. 5. The relationship between *N. glauca* height (m) and diameter (cm) measured in flooded open (A) and enclosed plots (B).



### 3.4. Resprouting

In the long-term flood zone, 41% of trees had retained original growth. Of the remainder, which had died back; 57% and 5% of the trees resprouted from the base and branches respectively. In the short-term flood zone, 60% of trees had retained their original growth. Of the remainder 19% had resprouted from the base, 13% from the stems and 7% from the base and branches.

### 3.5. Height and diameter class distribution

The diameter class distribution pattern of *N. glauca* in both the long and short-term flood zone showed an inverted 'J' curve. The vigour, as measured by height and diameter class distribution of *N. glauca*, varied between zones. In the long-term flood zone there was an even distribution of height classes. By contrast, in the short-term flood zone height class reduced slowly towards smaller diameter size class. It was difficult to establish a height class distribution pattern of *N. glauca* in the flood zone (Fig. 6A and B).

### 3.6. Soil-seed bank

Soil-stored seed bank density varied significantly ( $p > 0.0001$ ) between flooded exclosed ( $856 \pm 428.12$ ) and flooded open ( $1936 \pm 968$ ) plots. No *N. glauca* seed was found in the control plots, confirming results for the tree density assessment (Fig. 7). Analysis of the interrelations between number of *N. glauca* stems found at the time of sampling and number of seeds (Fig. 8) recovered from the soil produced the equation:

$$\text{Number of stems} = 0.4036 + 24.935(R^2 = 0.97; p < 0.001). \quad (5)$$

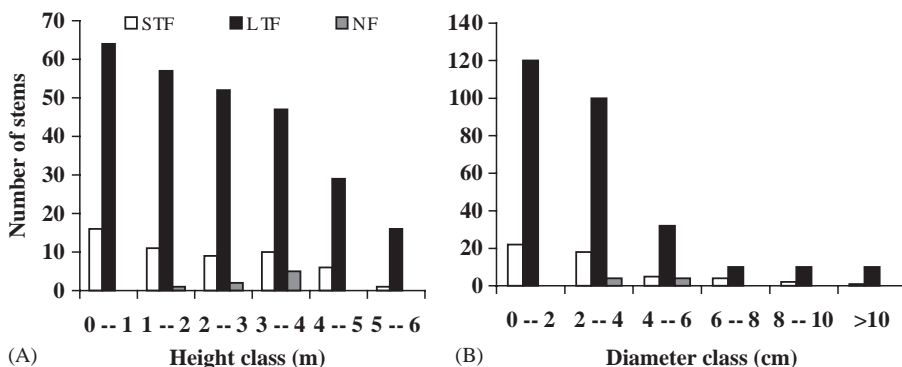


Fig. 6. Height (A) and diameter (cm) (B) distribution of *N. glauca* found in the Olary Creek. SFT short term flood zone; LTF long term flood zone and NF no flood.

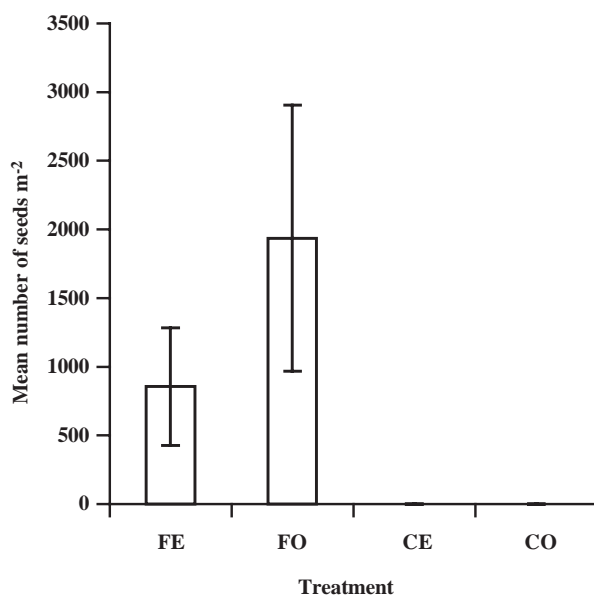


Fig. 7. *N. glauca* mean number of germinable seeds: FE flood enclosure, FO flood open; CE control enclosure and CO control open.

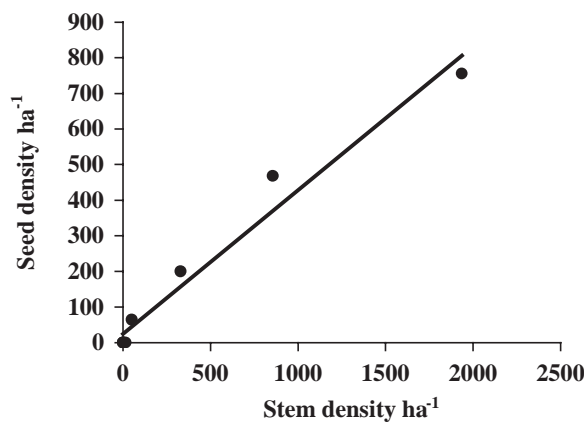


Fig. 8. The relationship between *N. glauca* stem density ha<sup>-1</sup> and seed density<sup>-1</sup>.

### 3.7. Allelopathic effect

Leachates from dry leaves and twigs significantly ( $p > 0.001$ ) decreased germination of *L. sativa* seeds. The effect was most pronounced with solutions of dry

leaf extract where in treatment 2,3,4, and 5 only 0, 0.28, 1.49 and 2.86% seeds germinated respectively, compared to 99% in the control. Similarly the twig extract gave a progressively greater effect as concentration was increased. In contrast, fresh leaves and bark caused no inhibition of *L. sativa* seed germination (Fig. 9).

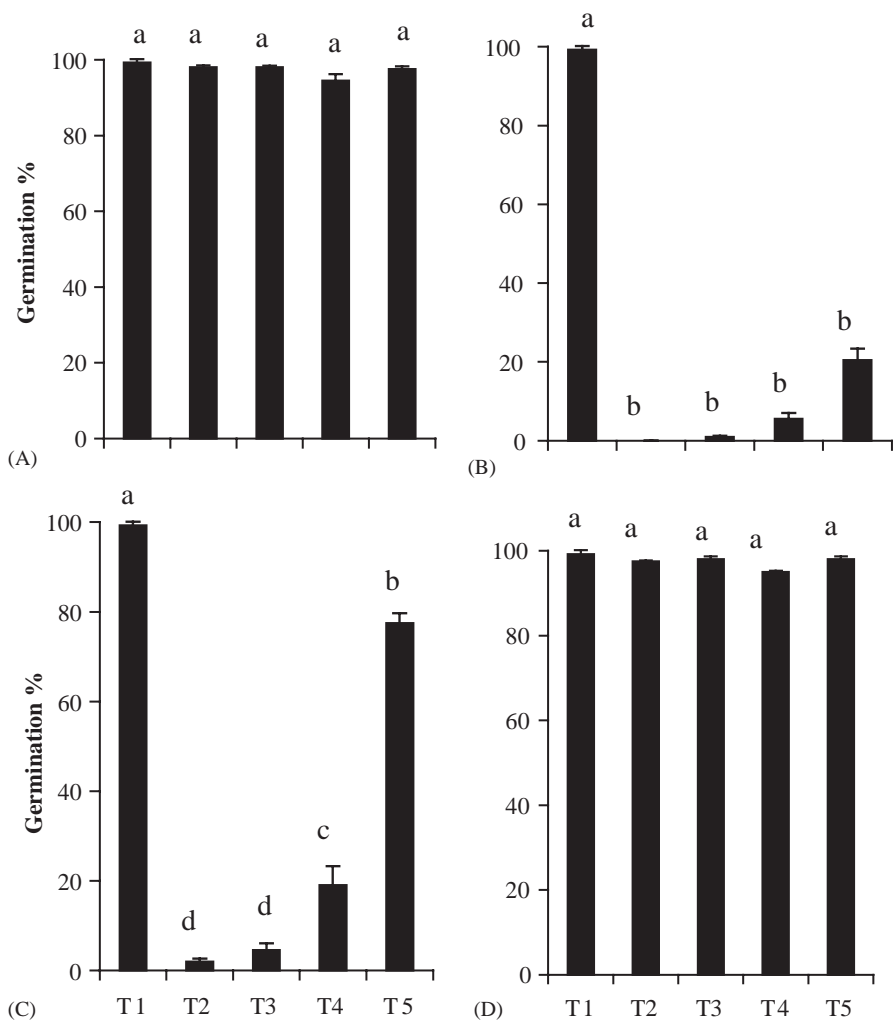


Fig. 9. *L. sativa* seed germination subject to *N. glauca* leachate. A green leaves leachate; B dry leaves leachate; C twig leachate, and D bark leachate. Treatment 1 (control) deionized water; Treatment 2 100% leachate; Treatment 3 75% leachate+25% deionized water; Treatment 4 50% leachate+50% deionized water and Treatment 5 25% leachate+75% deionized water. Columns topped by the same letter are not significantly different ( $p < 0.05$ ) as determined by Tukey's pairwise comparison. Vertical bars indicate standard error.

#### 4. Discussion

In this study, we examined: *N. glauca* tree density variation in flood and control plots; and between immediate flood, flood and sand dune zones. We also examined *N. glauca* soil-stored seed bank density and allelopathic impacts on *L. sativa* seed germination. *N. glauca* tree density varied across the plots and in relation to flooding zones. Larger numbers of *N. glauca* were found in the long-term flood zone than the short-term flood zone. No plants were recorded from areas not subject to flooding. Leachates obtained from dry leaves and twigs significantly decreased germination rate of *L. sativa* seeds whereas leachates obtained from fresh leaves and bark had no significant effect.

Weeds can cause detrimental effects on ecosystems by producing more seeds, occupying large productive areas, changing soil pH and hindering native flora by releasing allelopathic substances (Florentine, 1999). However, our study shows that density of *N. glauca* varied according to the disturbance. These differences between plots deserve special attention. Firstly, why were no *N. glauca* trees found in either the control fenced or unfenced plots? A possible explanation is, as has previously been reported, that *N. glauca* tends to colonize where there is high soil moisture and declines away from creeklines. The area around the new lake consists of well-drained sand with an open ground cover of *Triodia scariosa* ssp. *scariosa* and a sparse canopy of the *Eucalyptus dumosa* and *E. oleosa*. Secondly, why is *N. glauca* density nearly three times higher in the unfenced plots than fenced plots? The possible reason is grazing pressure from goats, kangaroos and rabbits. This high grazing pressure may have eliminated competition from native shrubs and grass species, creating ideal conditions for *N. glauca* seedlings to utilize available resources such as water and space to establish and survive during the subsequent summer months.

The variation in *N. glauca* density confirms that this species establishes in areas of high soil moisture. It is believed that the high rainfall event, resulting in flooding sufficient to leave standing water for several months, created ideal conditions for *N. glauca* establishment. Seeds of *N. glauca* are likely to have been brought from upstream and collected in the new lake. As flooded areas dried out, *N. glauca* seed deposited on peripheral mud (short-term flood zone) germinated and formed a dense stand. As Boyland et al. (1985) reported, *N. glauca* is a fast growing tree particularly after good rainfall events and produces flowers approximately one year after germination. *N. glauca* trees in the short-term flood zone may have supplied seeds to the long-term flood zone. This is supported by the population structure of *N. glauca* which shows that significant numbers of trees in the larger height and diameter class were found only in the short-term flood zone (Fig. 6).

The soil-stored seed bank of *N. glauca* reflects the density of stems present within respective plots and this was further confirmed by correlation analysis, showing a strong relationship. A patchy distribution of soil-stored seed is a common phenomenon for weed seeds. Although our study shows that a large quantity of seed is stored in the soil, the dynamics and longevity of small seed is unknown. From

the methodology used here it cannot be said whether seeds germinated from the soil seed bank are transient (seeds germinate soon after they are shed) or persistent (seeds can remain dormant in the soil for several years (Thompson and Grime, 1979; Simpson et al., 1989)).

When using bioassays to examine the allelopathic effects on germination, it is prudent to examine different concentrations of leachates (Martin and Smith, 1994). None of the concentrations of fresh leaf and bark leachates had any impact on *L. sativa* seed germination. This may be a result of *N. glauca* leaves being covered with a thick wax layer preventing phenolic compounds oozing from leaves or that soaking for twenty-four hours may not be sufficient time for leaves and bark to release the compounds. In contrast, leachates obtained from dry leaves and twigs caused a significant ( $p > 0.001$ ) reduction in germination of *L. sativa* seeds. As González et al. (1995) pointed out, allelopathic effects can be more pronounced during decomposition. However, extrapolation from controlled experiments to field conditions should be done with caution, since a number of biotic and abiotic factors may interact with each other in the field to influence the results (Florentine and Westbrooke, 2003). It is notable that *N. glauca* adds leaves and small twigs to the soil at the end of the dry season. Martin and Smith (1994) found a high proportion of phenolic or toxic compounds located in shallow (~5 cm) soil where seeds tend to germinate. The accumulation of leaves and small twigs on the soil surface may release toxic substances after a small amount of rainfall, the time most native species germinate. Newly emerged radicles are very sensitive to allelochemicals (Florentine, 1999), and high concentrations can easily kill new recruits. Further, since this species tends to colonize along creek-lines, dry leaves and twigs can also be transported and deposited downstream, with detrimental effects on downstream vegetation. Dendrochronology confirms that *N. glauca* invaded Olary Creek, and adjacent mallee shrubland following the 1997 flood event. It appears likely that seeds travelled during the flood event and were deposited along the banks and further into surrounding shrublands.

## 5. Conclusion

*N. glauca* is potentially a serious weed in arid catchments such as Olary Creek. Our study suggests that this species has traits that are comparable with other arid zone weed species. *N. glauca* tree density is significantly higher in the control plots where grazing has eliminated competition from pre-existing shrub and grass species. Native species are already facing increased grazing pressure from native and exotic animals (Westbrooke et al., submitted) and drought. Invasion by weed species such as *N. glauca* may put further pressure on their survival. Further research is required, particularly on flowering and fruiting phenology, seed production and viability and impact of fire on soil-stored seed-bank density.

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